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BIOCHEMICAL AGENT IDENTIFICATION: HIGH AFFINITY BINDING AGENTS AND MULTIPLE ELECTRONIC/PHOTONIC SENSORS

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14. ABSTRACT
A critical need exists for technologies to be employed as sensors for the rapid identification of a multiplicity of potentially harmful toxic agents in the environment. To this end, the Air Force Research Laboratory performed research to incorporate into or onto suitable substrates polymer conjugates that can bind to chemical, biological or other toxic agents. These multi-agent sensors present the potential for a real-time probe to enable the sensing and identification of known chemical and biological warfare agents as well as new agents for which no identification systems exists. This report documents the chemistry research performed to identify and configure the substrate polymer(s).

15. SUBJECT TERMS
Biological warfare agents, substrate polymers, biological sensors, biological threat agents

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1. SECTION I: Introduction, Summary and Conclusions

1.1 Introduction

Chemical and biological weapons remain a substantial threat to the national security of the United States. The Gulf War of 1991 was the most recent major instance of the threat by a foreign power to use chemical and biological weapons on a large tactical scale. The introduction of Anthrax (*Bacillus anthracis*) in the US Postal Service System by unknown persons in the fall of 2001 is an example of how the introduction of a single biowarfare agent can inflict hazards to the civilian population. In that instance the first isolation of the organism was made from the index human case in Florida and subsequent isolations were made via conventional microbiology methods from other human cases and buildings. Conventional microbiological identification methods are generally slow (hours to days). There remains a critical need for technologies to be employed as sensors to rapidly identify a multiplicity of possibly harmful toxic agents in the environment.

1.1.2 Concept, Scope and Process of the effort

Under contract with the Air Force Research Laboratory, Conceptual MindWorks, Inc. (CMI), performed research, planning, and integration necessary to incorporate into or onto suitable substrates polymer conjugates that can bind to chemical and biological or other toxic agents. These multiagent sensors pose the possibility of a real-time probe for the sensing and identification of known chemical and biological warfare agents, and of new agents for which there are no identification systems. The work reported herein includes the chemical research necessary to identify and configure the substrate polymer(s).

This effort explored the binding of the diazoluminomelanin (DALM) family of polymers (including synthesizing chemical derivatives) to proteins and to DNA molecules and explored methods of incorporating these polymers on various substrates. The process of the research included the synthesis and isolation of DNA oligomers selected for binding to potential biological warfare agents and subunits of such agents, including but not limited to specific proteins, nucleic acids, carbohydrates, and lipids associated with such agents. The possible spectral changes in the fluorescence of these polymers when they contact and bind with chemical and biological toxins and agents were also explored.

1.2 Executive Summary and Conclusions

This technical report describes the chemistry to attach DNA aptamers to beads and diazoluminomelanin (DALM) to the DNA. The authors have demonstrated that rDALM is able to quench fluorescent beads, whether attached directly to the bead or attached to the DNA, which is covalently linked to the bead. Also, dequenching has been demonstrated when the DNA double strands are denatured by heating (which simulates

binding of a target molecule) and the DALM removed from the beads. Finally the DNA on beads can be amplified through the polymerase chain reaction, but unfortunately once DALM was attached to the DNA, a different pattern of PCR products was found. Thus, rDALM besides quenching fluorescence also quenches the amplification of DNA by the polymerase chain reaction. The major part of this system still to be demonstrated is that the strand of DNA bound to the bead can be amplified by PCR after removal of the complimentary DNA strand attached to the rDALM upon either strands binding the target.

These results serve as the basis for a method to rapidly select single stranded DNA aptamers. To start, a library of random pieces of double stranded DNA of defined length (40 to 60 bases), with know ends (of 12 to 20 bases, primers, required for PCR amplification) are attached to fluorescent magnetic beads. The goal is to have, on average, one aptamer per bead. To the aptamer, rDALM will be attached which will quench the fluorescence of the beads. These beads will then be incubated with the target, a small molecule, a protein, a viral particle, or a microorganism. Binding of the target to one strand of the double stranded piece of DNA will "melt" or denature the double stranded DNA. This will remove the rDALM from the beads, dequenching them and resulting in fluorescence. Those beads that have DNA, which can bind to the target, will thus fluoresce. The beads will be passed through a magnetic bead sorter. Magnets will separate beads that fluoresce from those beads that fail to fluoresce. The single stranded DNA on these fluorescent beads will be amplified by the polymerase chain reaction. Note, it does not matter which strand actually binds the target, either the strand attached to the bead (which is the one that starts the amplification process) or the strand bound to the rDALM. After amplification, it can be determined which strand binds the target molecule. If necessary, at this point the process could be repeated by linking the DNA amplified from the fluorescent beads to beads again and repeating the separation procedure. Also, the DALM besides quenching the fluorescence also quenches the amplification of DNA by the polymerase chain reaction. The major part of this system still to be demonstrated is that the strand of DNA bound to the bead can be amplified by PCR after removal of the complimentary DNA strand attached to the rDALM upon either strands binding the target.

2. SECTION II: Synthesis of DALM and its derivatives

Abbreviations

DALM: Diazoluminolmelanin
EDC: 1-ethyl-3-(3 dimethylaminopropyl) carbodiimide HCl
EDA: Ethylenediamine dihydrochloride
3AT: 3-aminotyrosine
MOPS: 3-(N-morpholino) propanesulfonic acid or 3-Morpholinopropanesulfonic acid

Reduced DALM (rDALM)

Dissolve 1.596 g of luminal with stirring in 150 mL of dimethylsulfoxide in a 500 ml-round bottom flask. Dissolve 2.664 g of 3-aminotyrosine in 150 ml of water, add 0.621 g of sodium nitrite to the 3AT solution, wait four minutes and add the diazotized 3AT to the luminal while stirring. Reflux the solution for 6 hours. Allow the reaction to cool to room temperature by stirring over night.

The next morning transfer the reaction to a 2 L-separatory funnel containing approximately 1.6 L of acetone. The rDALM will precipitate and settle out over night. The crude rDALM is collected from the bottle of the funnel followed by centrifugation. The crude rDALM is dissolved in an aqueous ammonia solution (1 ml of 37% ammonia in 20 ml of water) and transferred to a dialysis bag with a 3,500 Dalton cut off. The crude rDALM is dialyzed against several changes of a dilute aqueous ammonia solution (5 ml of 37% ammonia up to 2 L of water) and then against water. Lyophilizing dries the rDALM.

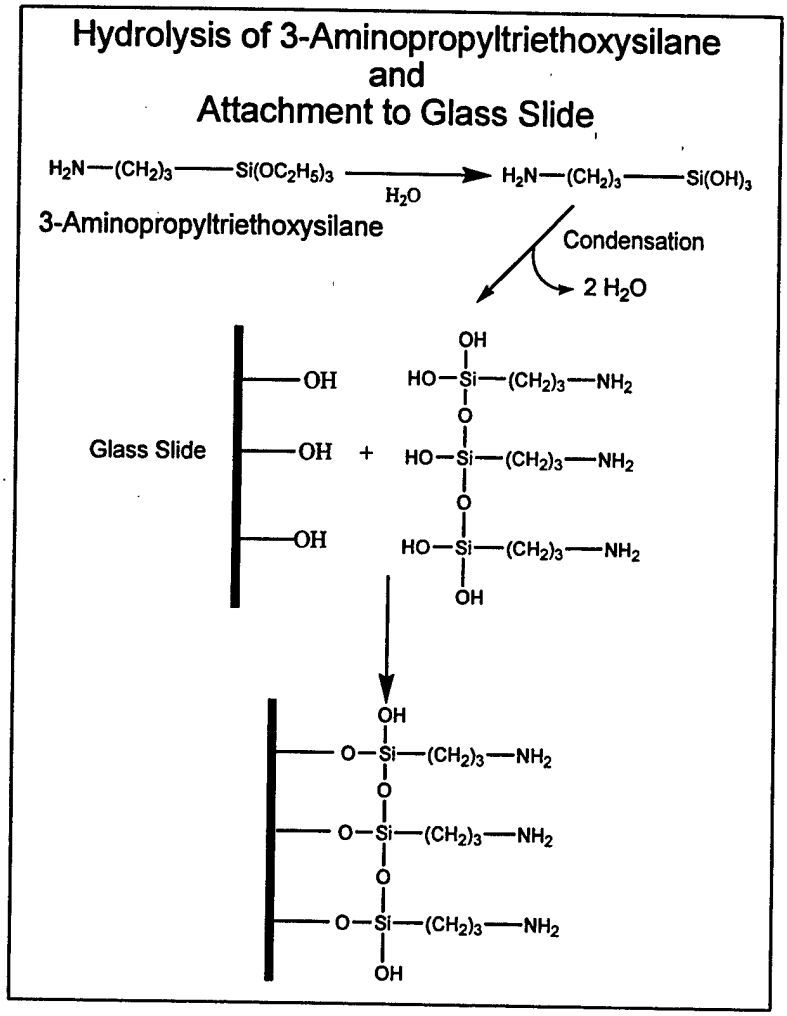
Nitro-DALM (nDALM)

Peroxynitrite: Prepare 1 M $NaNO_2$ by dissolving 1.38 g of sodium nitrite in 20 ml of water. Prepare 1.4 M NaOH by dissolving 1.12 g of sodium hydroxide in 20 ml of water. Prepare 1 M H_2O_2 in 0.3 M H_2SO_4 by adding 2.27 g of hydrogen peroxide in water, add 0.33 ml of concentrated sulfuric acid, and bring the volume of water up to 20 ml. Cool all solutions on ice. When cool, transfer the sodium nitrite solution to a chilled 250-mL beaker in an ice bath. Stir the solution. Add the hydrogen peroxide-sulfuric acid solution to the beaker, and within a second or two add the sodium hydroxide solution to quench the reaction (the synthesis of peroxynitrite is acid catalyzed as is its rearrangement to nitric acid; however it is stable in base). While stirring the solution, add solid MnO_2 to destroy the remaining hydrogen peroxide. To ensure no hydrogen peroxide remains, add an additional pinch of MnO_2. If no bubbling occurs, filter the solution using a 0.2-micron filter. Transfer the yellow solution to a 50-mL centrifuge tube, and keep cool on ice. Determine the concentration of the peroxynitrite solution by using the molar extinction coefficient of $1.67 \times 10^3\ M^{-1}$.

A typical preparation of nDALM follows. Transfer 174 mg of rDALM to a 100-mL round bottom flask. Add 30 ml of 82-mM peroxynitrite and reflux for four hours while stirring. The solution is allowed to cool to room temperature and the remaining peroxynitrite solution is added to the round bottom flask and the reaction mixture stirred over night at room temperature. The next morning, prepare an additional 50 ml of peroxynitrite and add it to the round bottom flask. Stir at room temperature for an additional 24 hours. The next morning the solution is dialyzed against water using dialysis tubing with a 3,500 Dalton cut off. Dialyze against several changes of water, and lyophilize to dryness.

Oxidized DALM (oDALM)

A typical synthesis of oDALM follows. Dissolve 481 mg of rDALM in 50 ml of water containing 0.8 g of sodium carbonate. Add 5 ml of 30% hydrogen peroxide. Reflux at as lowest heat as possible. Every 30 minutes add 2 ml of 30% hydrogen peroxide (do this 7 times). Allow the solution to cool to room temperature and add 5 ml of 30% hydrogen peroxide and stir over night. The reaction is complete when the solution is clear and yellow. In some cases it maybe necessary to add 0.1 g of sodium bisulfite with refluxing for 30 minutes to re-reduce the oxidized DALM. This is only required if it is necessary that the oDALM slow fluoresce.



The oDALM is dialyzed against several changes of water using 3,500 Dalton cut off dialysis tubing and lyophilize to dryness.

Immobilization of DNA and DALM

DALM seems to have no available amino groups, but it does have available carboxylate groups. The general outline of the chemistry used was to activate carboxylate groups with EDC and link them to amino groups through an amide bond. DNA may also be linked to amino groups through similar chemistry, with EDC activating either the 3' or 5' phosphate and reacting with an amine to form a phosphoramide.

Immobilization of DALM on Glass Slides

Glass slides, either frosted or unetched were cleaned (and etched) over night using alcoholic KOH (950 mL absolute ethanol, 170 ml of water, 105 g KOH). Slides were washed with distilled water and allowed to air dry. Silyating agent was prepared by

mixing 12 mL of 3 aminopropyltriethoxysilane, 8 ml of water, and 180 ml of acetone. The slides were placed in this solution and shaken in this solution for two hours. The slides were rinsed in acetone and air-dried. Slides were either treated with rDALM or oDALM. For rDALM, suspend 150 mg of rDALM in 0.1 M MOPS, pH 7 and add 2 drops of 10g/100mL NaOH for it to dissolve. For oDALM, the NaOH is not added; it dissolves fine in MOPS at pH 7. Add enough MOPS for the solution to cover the slides. Place the slides covered by the rDALM solution on an orbital shaker and add EDC. For the first two hours add approximately 200 mg of EDC every 15 minutes. Then add 300 mg of EDC. Shake for an additional 2 to 3 hours. Pour off the reaction mixture and wash the slides several times with water. The slides are allowed to air dry.

Immobilization of DALM and DNA on beads

Transfer 1.5 ml of Fluoresbrite Carboxylate Microspheres (2.64% solids-latex, catalog # 19395, diameter 5.703 microns) to a 15 ml (17 X 100 mm Polypro) centrifuge tube. Collect beads by centrifuging (5 minutes at 3,000 rpm in a bench top clinical centrifuge with a swinging bucket rotor). Decant the supernatant and wash the beads twice in 3 ml of 0.1 M MOPS buffer, pH 7. Beads are resuspended in 2.0 ml of MOPS buffer.

Below are calculations on the minimum amount of EDA and EDC to use. Polysciences informed me that the beads have 0.2 millimoles of carboxylate groups per gram of beads.

$$\text{Moles of COOH groups} = 1.5\ \text{mL}\left(\frac{2.64\ \text{g of beads}}{100\ \text{mL of beads}}\right)\left(\frac{2 \times 10^{-4}\ \text{mols COOH}}{1\ \text{g of beads}}\right)$$

$$= 7.92 \times 10^{-6}\ \text{moles}$$

$$\text{Grams of EDA·2HCl} = 10\ \text{fold excess}\left(7.92 \times 10^{-6}\ \text{mols COOH}\right)\left(\frac{133.04\ \text{g}}{\text{mole}}\right)$$

$$= 0.0105\ \text{g}$$

$$\text{Grams of EDC} = \left(\frac{50\ \text{mols EDC}}{1\ \text{mol COOH}}\right)\left(7.92 \times 10^{-6}\ \text{mols COOH}\right)\left(\frac{191.7\ \text{g EDC}}{\text{mol EDC}}\right)$$

$$= 0.0759\ \text{g}$$

The carboxylate groups are converted to amino groups by reacting them with the bifunctional agent EDA at room temperature. EDC activates the carboxylate groups, allowing the formation of an amide bond. A typical reaction used 24.4 mg of EDA dissolved in 1 ml of 0.1 M MOPS buffer, pH 7.0. The EDA solution was added to the 2.0 ml of washed beads. A large excess of EDC was added as well as additional EDA according to the following addition scheme was then followed.

Time	mg of EDA	mg of EDC	ml of MOPS buffer
0940 hrs	40.7	117	1.0
1000 hrs		95	
1020 hrs		85	
1040 hrs	48.9	100	1.0
1100 hrs		80	
1120 hrs		114	1.0
1140 hrs		97	
1240 hrs	53.6	210	1.0
1340 hrs		102	

At 1615 hrs the beads were collected by centrifugation and washed several times with water. The beads were resuspended in 2.0 ml of MOPS buffer and stored overnight in a refrigerator.

An AFRL/HE researcher provided plasmid DNA cut by restriction enzymes. The night before, 0.2 ml of MOPS buffer was added to each microcentrifuge tube, and the DNA was allowed to incubate refrigerated in the buffer overnight. The next morning the DNA was pooled in a small reaction vessel. The concentration of the DNA was 129 □g/ml and there was 1 ml of DNA. Assuming the length of the average DNA piece was 1 kilobase, the number of moles of DNA pieces was

$$= 130 \times 10^{-6}\ g\left(\frac{1}{1{,}000\ bp}\right)\left(\frac{1\ bp}{660\ g/mole}\right) = 1.97 \times 10^{-10}\ moles.$$

Add 0.3 ml of beads to the DNA. EDC and additional MOPS buffer were added according to the schedule that follows.

Time	mg of EDC	ml of MOPS
1020 hrs	103	1.0
1100 hrs	120	
1140 hrs	105	1.0
1220 hrs	108	
1300 hrs	133	1.0
1340 hrs	112	

Collect the beads at 1600 by centrifugation. The beads are washed several times in DNA buffer and stored refrigerated over night in 1 ml TAE buffer. Save a 100 □L aliquot for PCR analysis. In the morning, transfer the beads to MOPS buffer by collecting them by centrifugation and resuspending them in 1 ml MOPS.

Preparation of amino-DALM. Dissolve 46.1 mg of rDALM in 3 ml of 0.1 M MOPS buffer, pH 7. Add 61.0 mg of EDA in 1 ml of MOPS buffer. Additional EDC and EDA were added as follows

Time	mg of EDC	mg of EDA	ml of MOPS
1000 hrs	91		
1020 hrs	119		
1040 hrs	91	1.0	
1100 hrs	92	81	1.0
1330 hrs	51		
1415 hrs	104		

The reaction mixture was dialyzed against 2 L of water using 3,500-dalton cut off dialysis tubing. After several changes of dialysis water, the amino-DALM was placed in an ultrafiltration column with a YM3 membrane (3,000 dalton cut off). Water was added several times. This was to ensure that no unreacted EDC or EDA remained. The amino-DALM was lyophilized to dryness. Weight of amino-DALM was 36.3 mg; probably no unreacted EDC or EDA remained.

The amino-DALM was dissolved in 4 ml of 0.1 M MOPS buffer, pH 7.0. The beads were added and EDC was added according to the following schedule

Time	mg of EDC
1020 hrs	100
1105 hrs	122
1145 hrs	145
1220 hrs	102
1300 hrs	136

Two hours after the final addition, the beads were collected by centrifugation and washed in TAE buffer. Wash beads twice in 0.5 M Tris, pH 9.5 buffer to remove any non-covalently bound amino-DALM. The wash was colorless. Beads were washed again in TAE and then resuspended in 1 ml of TAE buffer. A 100 ml aliquot was saved for PCR analysis.

The remaining beads were boiled for 20 minutes, and immediately cooled on ice. The beads were again collected by centrifugation and washed several times in TAE buffer. The wash was colorless and the beads were still brown. An aliquot was provided for PCR analysis.

The advantage of synthesizing amino-DALM was that the beads did not need protection. Before recognizing this advantage, unreacted amino groups on the beads were protected by acetylation or reacting with calf-thymus DNA. The example given did not require any protection and avoided exposing the DNA to EDA, which might cross-link the DNA.

3. SECTION III: Results and Discussion

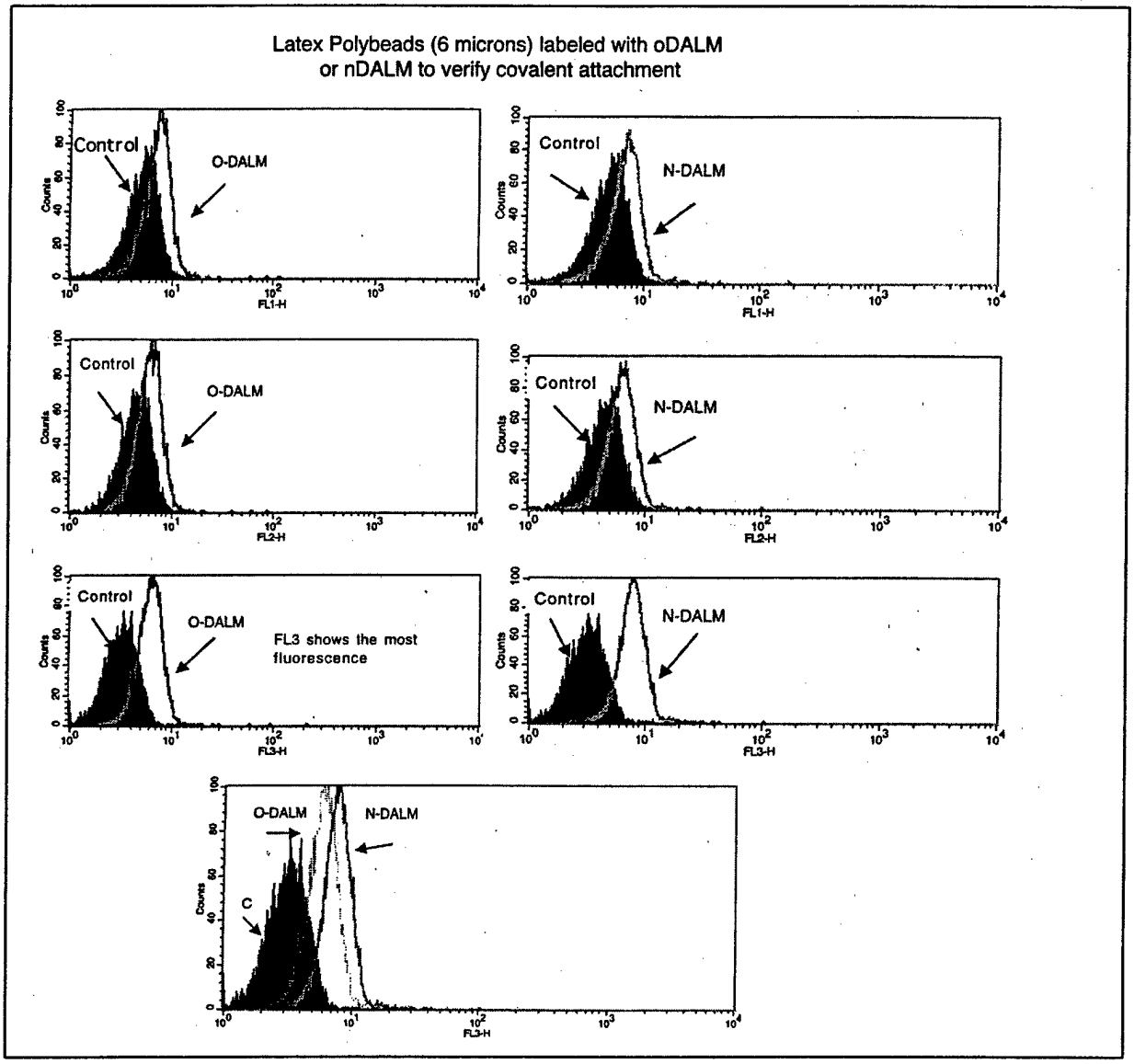
Immobilization on Glass Slides

DALM was originally immobilized on clear glass microscope slides. The slides had a brown tint, but since rDALM was used it was not possible to measure fluorescence. In order to increase the amount of surface area available for immobilization, frosted slides were used in some experiments. Again the brown tint of rDALM could be seen on these slides. When oDALM was attached to the slides, they picked up a yellow tint, and with the use of hand-held ultraviolet lights a blue-green fluorescence could be seen. Unfortunately, because of scatter, we were unable to collect any idea on these slides using the PTI spectrofluorometer. In talking with PTI, they suggested using filters to cut off the incident light and a better slide or solid sample holder. However, it was decided not to proceed with glass immobilization but to use microspheres or beads to immobilize DALM and DNA.

Immobilization on Latex Microspheres (or beads)

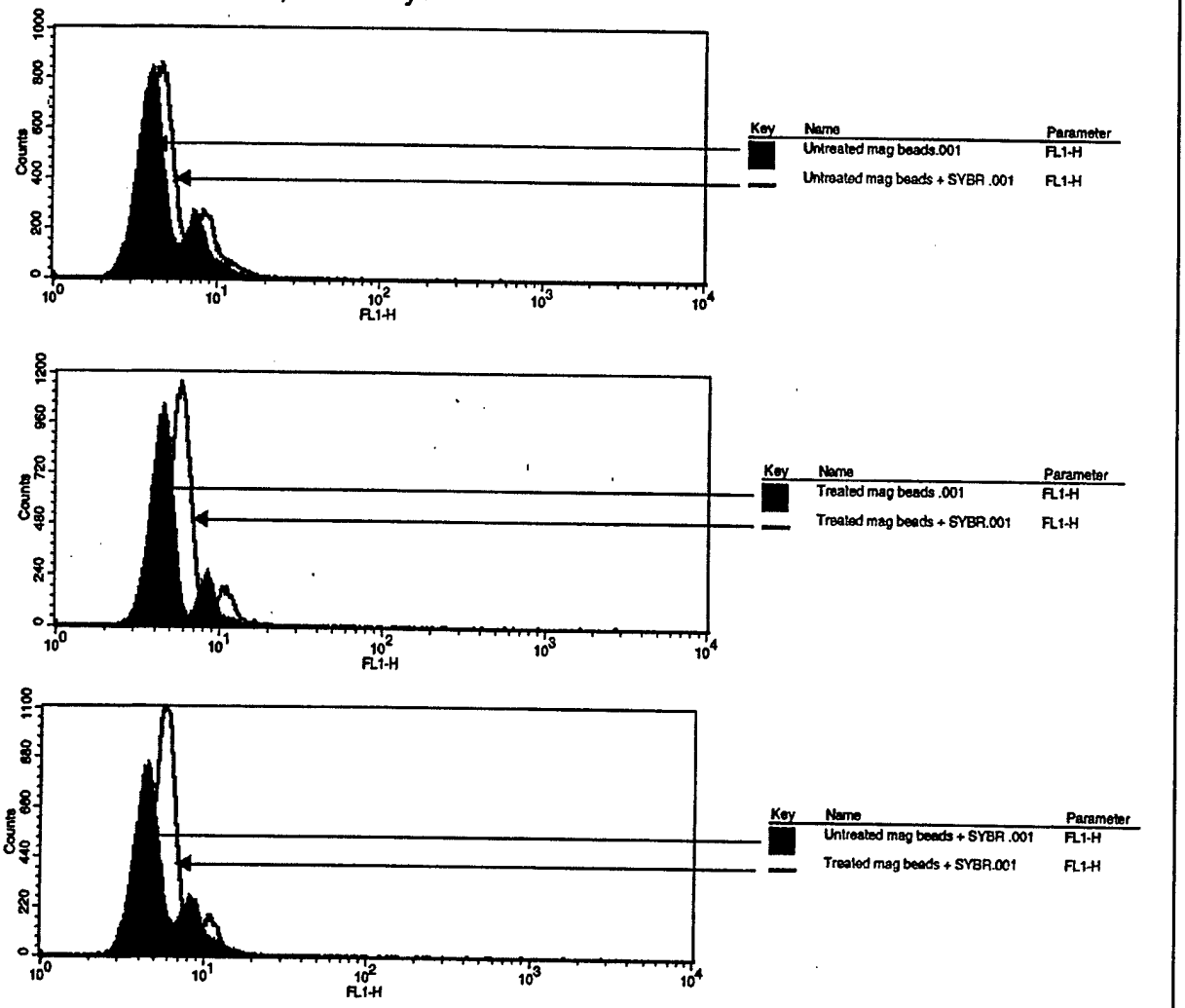
The original thought in using beads or microspheres was to simplify the synthesis of attaching DALM to DNA. The concern was isolating the DALM-DNA complex from unreacted DALM and DNA. Since DALM is notorious for its binding to chromatography media, that separation route was eliminated. It was believed that if the DALM-DNA complex could be grown on a bead, simple centrifugation or, if the beads were magnetic, a magnetic would suffice to separate the DALM-DNA complex from the reactants. The complex could then be hydrolyzed off of the bead at a latter time to yield pure complex. It was realized DNA binding to beads could be determined using a flow cytometer and fluorescent DNA dyes. Furthermore, the binding of oDALM to beads could also be determined by the fluorescence of oDALM using a flow cytometer. Eventually, we realized an entire detection system could be built around beads and flow cytometers.

Either oDALM or nDALM were reacted with Latex Polybeads (6□) by PolySciences as described in the experimental section. RDALM was not used because it doesn't fluoresce. The histograms below clearly demonstrate that the beads with oDALM or nDALM clearly fluoresce compared to control beads.



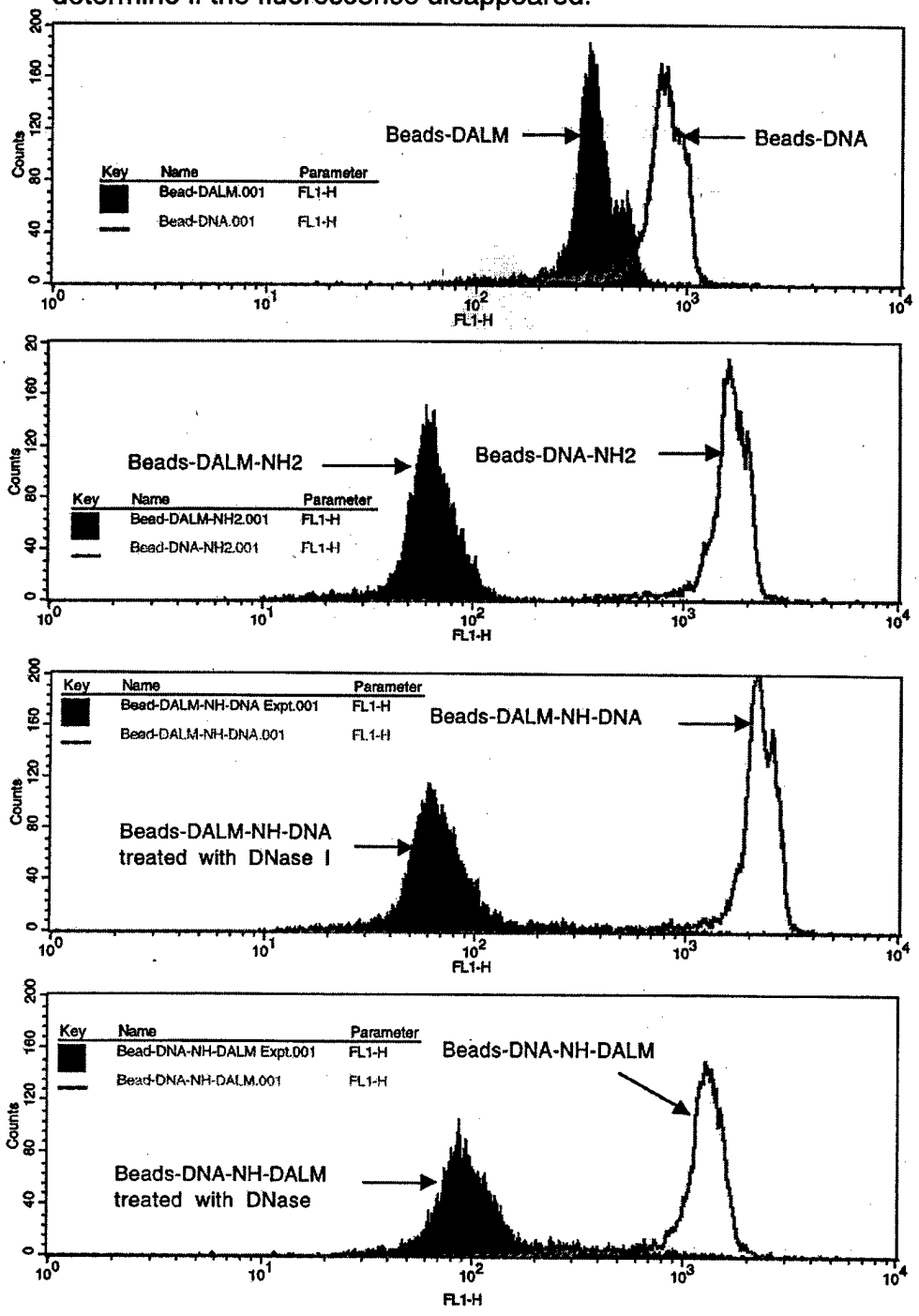
To verify that DNA could be attached to beads, amino deriviatized magnetic beads were used, and DNA attached using the chemistry described. As can be seen, in the following figure, the beads with DNA attached to them have an increase in fluorescence compared to the controls (treated beads contain DNA). The top histogram shows that some SYBR Green (a DNA dye) must bind to the magnetic beads, and increases fluorescence slightly. Beads with DNA and dye fluoresced more than beads with just DNA (middle histogram). The bottom histogram compares beads without DNA but exposed to SYBR Green to beads with DNA attached and exposed to SYBR Green. These histograms clearly indicate that DNA can be attached to beads by the chemistry in the experimental section, and that DNA detected.

Single Stranded DNA Aptamers on Magnetic Beads. Treated beads contain DNA. Beads with and without DNA were exposed to SYBR Green, a DNA dye.



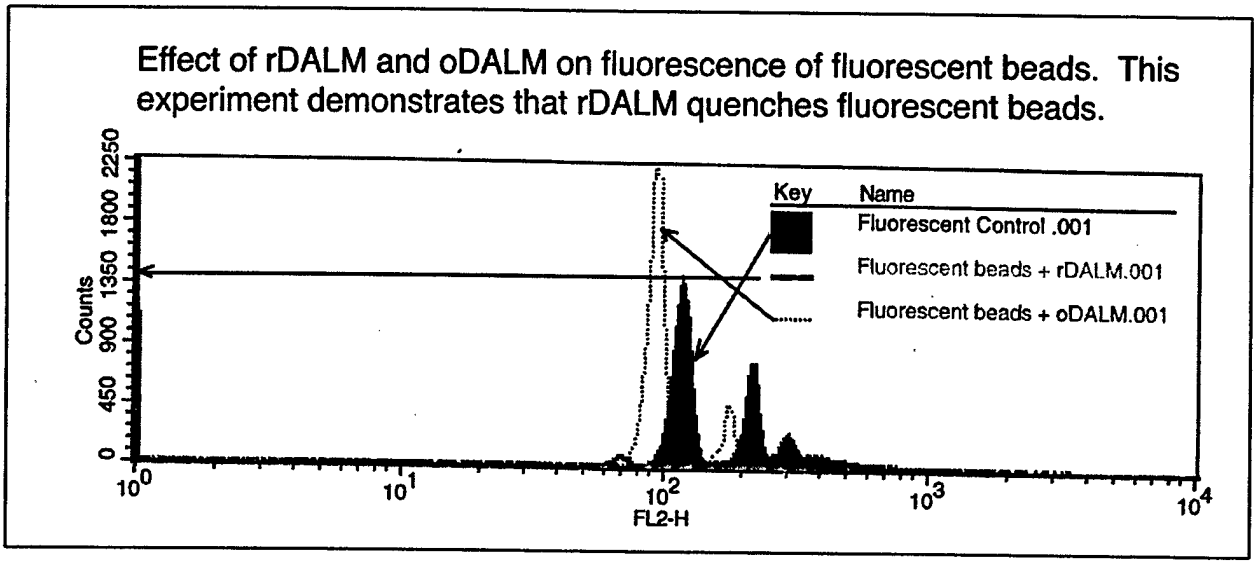
In the next experiment, in the top histogram, either DNA or oDALM are attached to latex beads, and SYBR-Gold added to the beads. Clearly the beads with DNA attached fluoresce considerably more than the beads with DALM. In the next histogram, EDA has been added to the ends of the macromolecules, and again the beads with DNA attached fluoresce more when SYBR-Gold is added. The third histogram shows the completely built DALM-DNA chain attached to the beads. These beads clearly fluoresce when SYBR-Gold is added, but the beads treated with DNase I no longer fluoresce when SYBR-Gold is added, indicating loss of the DNA. The bottom

Latex Beads with either DALM plus EDA attached or DNA plus EDA attached. SYBR-Gold, a DNA dye, was added to increase the fluorescence of the DNA. Beads were also treated with DNase I to determine if the fluorescence disappeared.

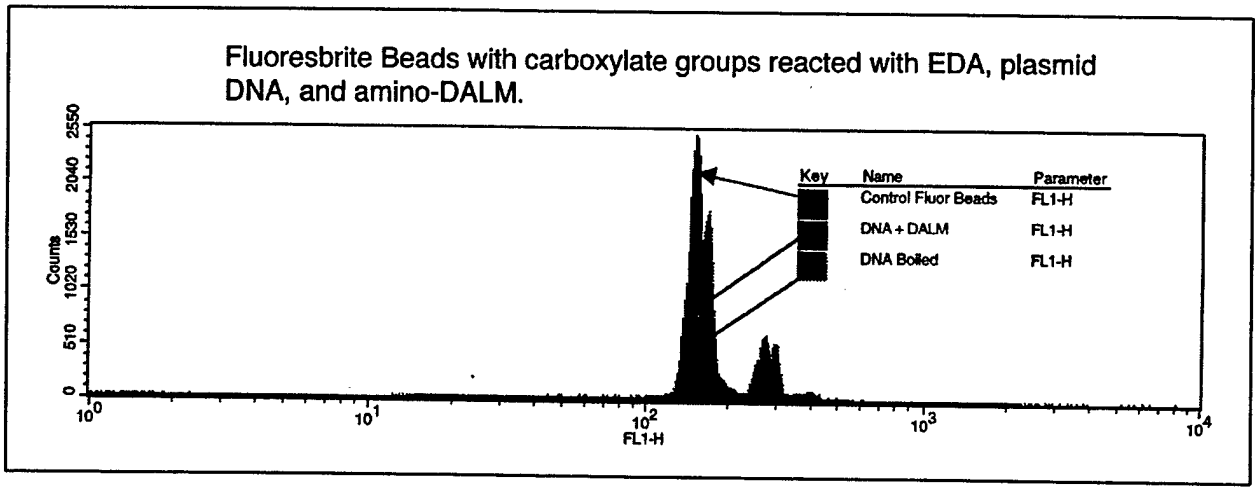


histogram has the order of the DALM/DNA reversed, with DNA next to the bead, the EDA linker next, and DALM on the outside. When SYBR-Gold is added to these beads, they clearly fluoresce, but when DNase I is added, which removes the DNA, the fluorescence is lost, since no DNA remains for the dye to react with and fluoresce.

These series of experiments demonstrate that DALM and DNA can be attached to beads and attached to each other on the beads. At this point, we decided to pursue a system that would be quenched until a target molecule bound. This required use of a fluorescent bead, and using DALM to quench the fluorescence. The following histogram demonstrates that rDALM quenches the fluorescent beads, but that oDALM does not.



Fluoresbrite beads with carboxylate groups were modified as described with EDA and EDC, and plasmid DNA attached. The DNA was then modified with EDA and finally reacted with rDALM. The method listed in the procedures section is a superior method in which amino groups are attached to the rDALM, and the DNA never sees the bifunctional agent EDA, which may cross link it and prevent the DNA from undergoing polymerase chain reaction. The following histogram indicates that rDALM quenches the fluorescence of the beads slightly. When the beads are boiled to simulate the binding of a target molecule which would cause the DNA strands to separate and free the bead from the DALM, there is an increase in fluorescence, as would be expected. These results support the model of dequenching to indicate a target has bound to the DNA.



Polymerase Chain Reaction Results

The polymerase chain reaction was performed on the beads at different steps. It was possible to obtain the expected band after the DNA was attached to the beads. But once rDALM was added to the DNA, it was impossible to obtain the expected DNA band. Its as if the rDALM quenched PCR. This was also true for the boiled sample. In this case DALM should not be present in the PCR reaction. There were two possible reasons. First, boiling removed the DNA from the bead, or that somehow EDA cross-linked the DNA.

The first possibility gave rise to the method of attaching EDA to the DALM, purifying the DALM, and then reacting this amino DALM with the DNA. Using this method, the DNA never sees EDA to be possibly cross-linked. Unfortunately, even in this case, the expected PCR band was not obtained from the DNA with DALM, either before boiling or after. However, a band was found in the agarose gels run on the PCR product from the DNA with DALM and beads that were boiled. This band was unique in that the beads did not give this band in absence of PCR reagents or enzymes. It is believed to be a true PCR produce, but not the one expected. It is unable to be determined the significance of this band. One would hope to be able to PCR the DNA on the beads after boiling (which is suppose to simulate the binding of a target molecule). This DNA is an aptamer and would permit this method to rapidly select aptamers, assuming some method of bead sorting was incorporated into the flow cytometer.

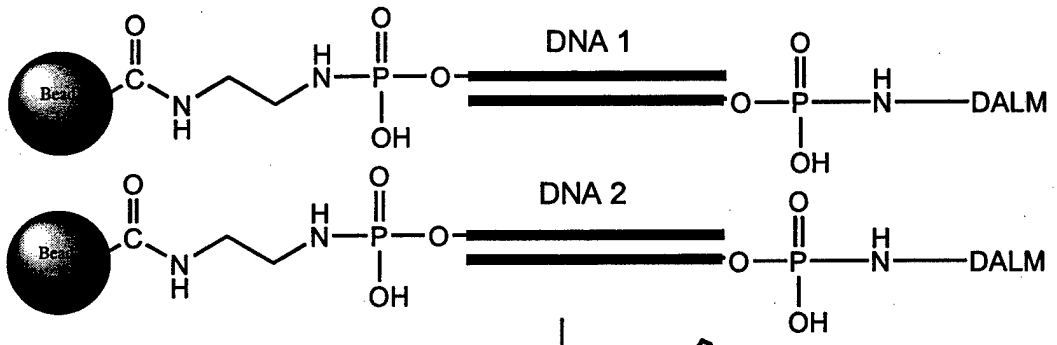
4. SECTION IV: Conclusions

Beads were originally intended as a synthetic tool, but they have become the basis of the goal to rapidly select DNA aptamers. The chemistry to attach DNA aptamers to beads and DALM to the DNA has been developed. It has been demonstrated that rDALM is able to quench fluorescent beads, whether attached directly to the bead or attached to the DNA, which is covalently linked to the bead. Also, dequenching has been demonstrated when the DNA double strands are denatured by heating (which simulates binding of a target molecule) and the DALM removed from the beads. Finally the DNA on beads can be amplified through the polymerase chain reaction, but unfortunately once DALM was attached to the DNA, a different pattern of PCR products was found. Thus, rDALM besides quenching fluorescence also quenches the amplification of DNA by the polymerase chain reaction. The major part of this system still to be demonstrated is that the strand of DNA bound to the bead can be amplified by PCR after removal of the complimentary DNA strand attached to the rDALM upon either strands binding the target.

These results serve as the basis for a method to rapidly select single stranded DNA aptamers. To start, a library of random pieces of double stranded DNA of defined length (40 to 60 bases); with know ends (of 12 to 20 bases, primers, required for PCR amplification) are attached to fluorescent magnetic beads. The goal will be to try to have, on average, one aptamer per bead. To the aptamer, rDALM will be attached which will quench the fluorescence of the beads. These beads will then be incubated with the target,

a small molecule, a protein, a viral particle, or a microorganism. Binding of the target to one strand of the double stranded piece of DNA will "melt" or denature the double stranded DNA. This will remove the rDALM from the beads, dequenching them and resulting in fluorescence. Those beads that have DNA, which can bind to the target, will thus fluoresce. The beads will be passed through a magnetic bead sorter. Magnets will separate beads that fluoresce from those beads that fail to fluoresce. The single stranded DNA on these fluorescent beads will be amplified by the polymerase chain reaction. Note, it does not matter which strand actually binds the target, either the strand attached to the bead (which is the one that starts the amplification process) or the strand bound to the rDALM. After amplification, it can be determined which strand binds the target molecule. If necessary, at this point the process could be repeated by linking the DNA amplified from the fluorescent beads to beads again and repeating the separation procedure. Also, the DALM besides quenching the fluorescence also quenches the amplification of DNA by the polymerase chain reaction. The major part of this system still to be demonstrated is that the strand of DNA bound to the bead can be amplified by PCR after removal of the complimentary DNA strand attached to the rDALM upon either strands binding the target. See the figure below for example.

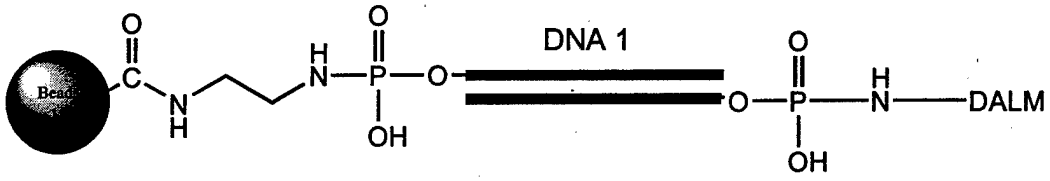
Example of how binding of target to single stranded aptamer creates a flourescent bead that can be separated and the DNA amplied by PCR

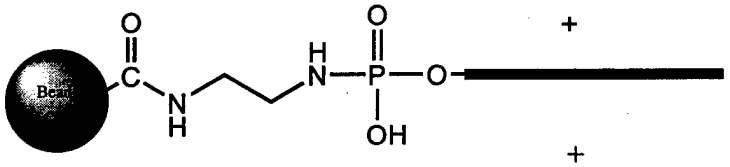


These beads are quenched

Protein Target

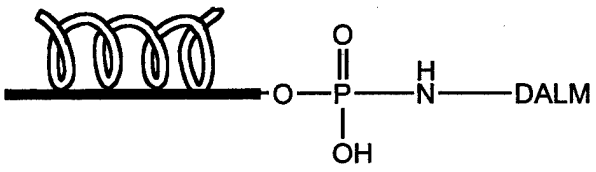
Quenched bead



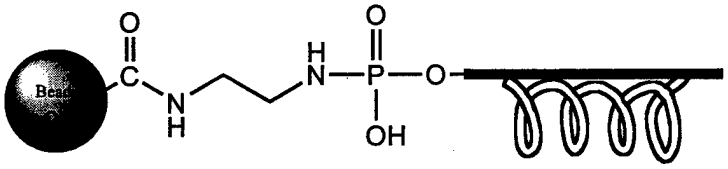


Bead Flouresces

Target bound to single strand of DNA

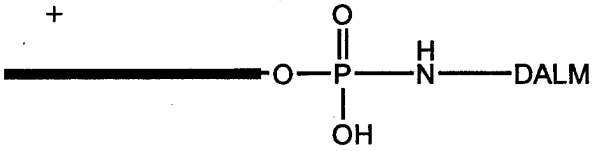


or



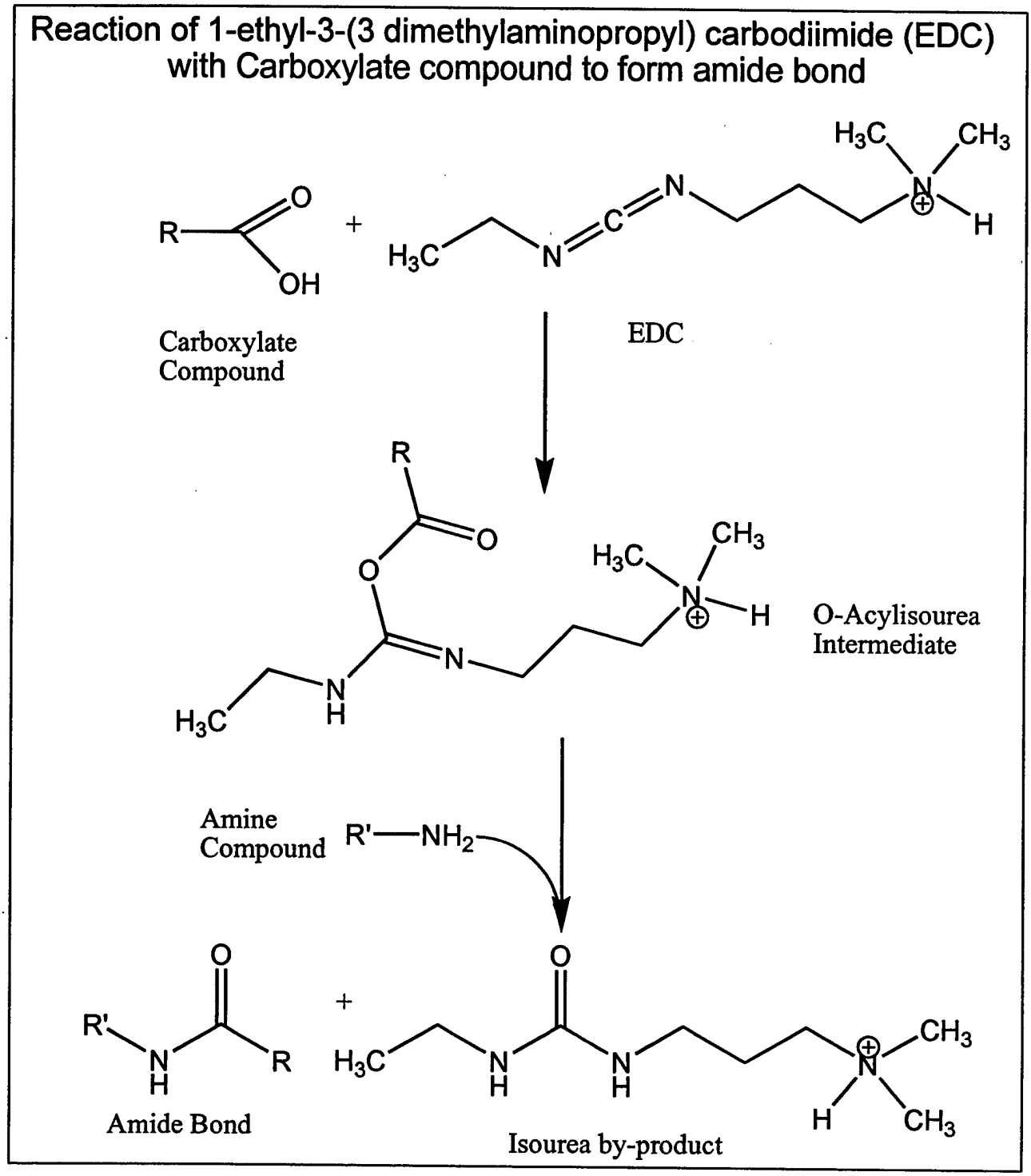
Bead Flouresces

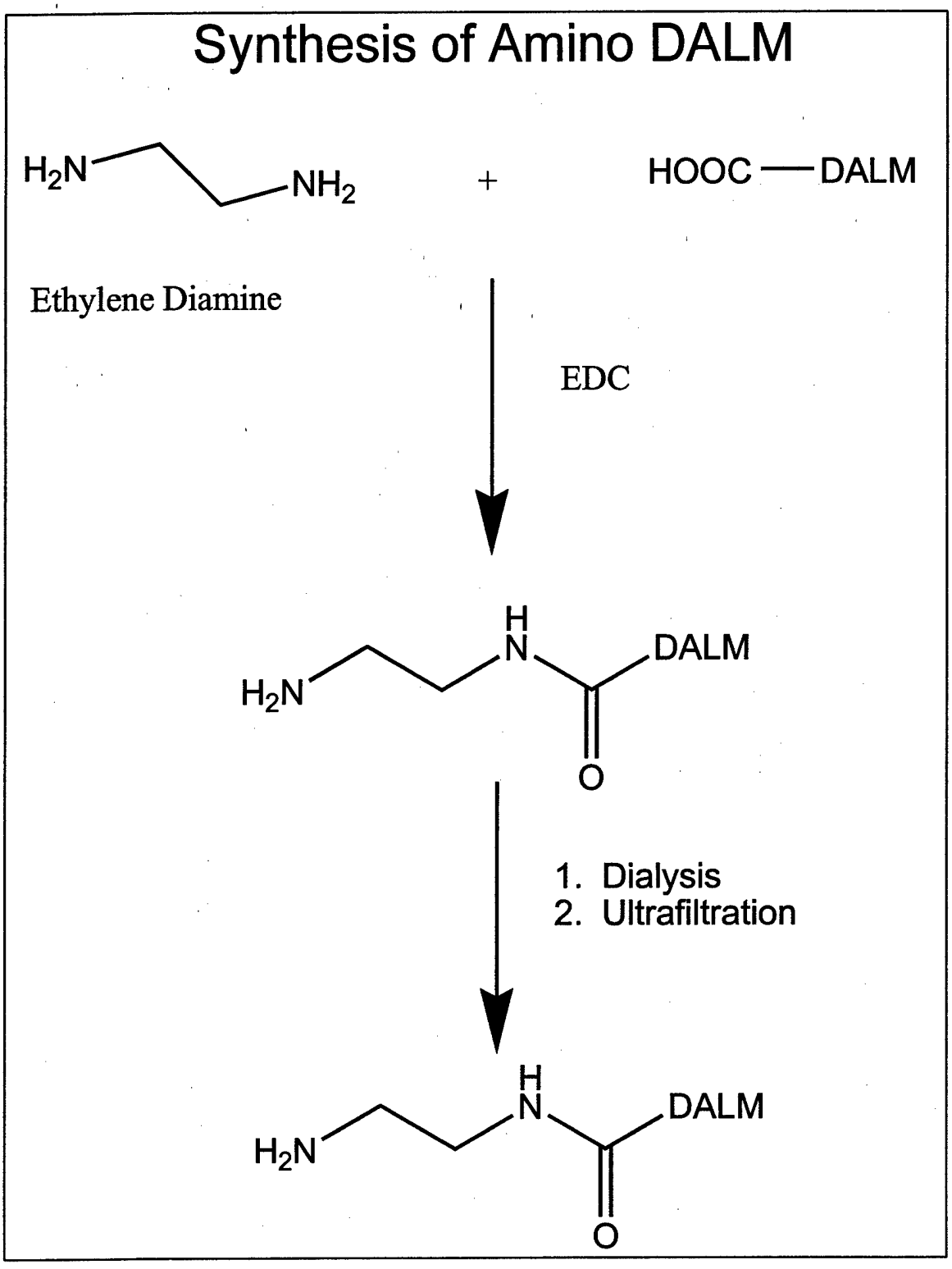
Note it does not matter which of the strands binds the target, the result is the same, the bead still fluoresces and the information needed to find the sequence is still there.

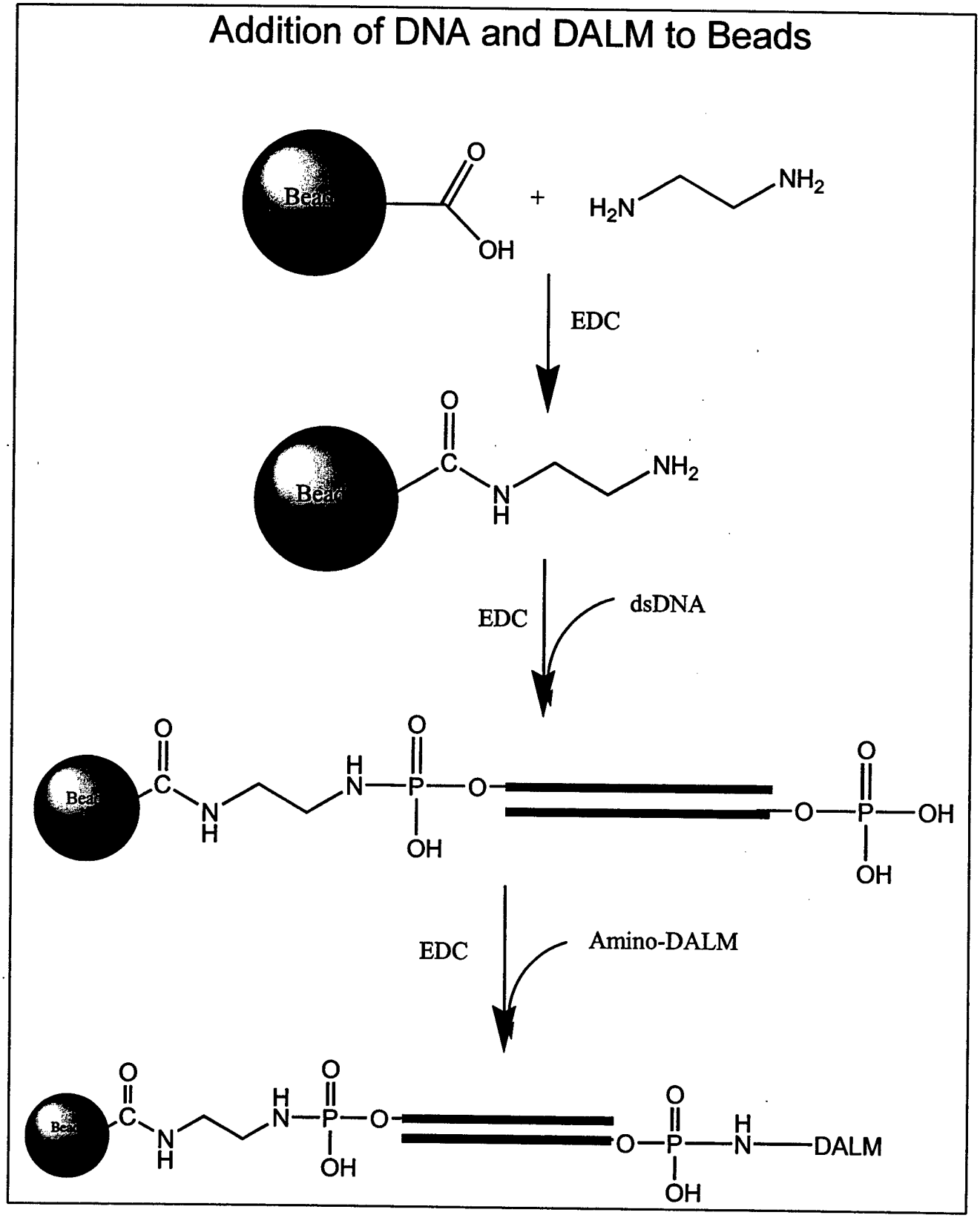


5. SECTION V: Appendices

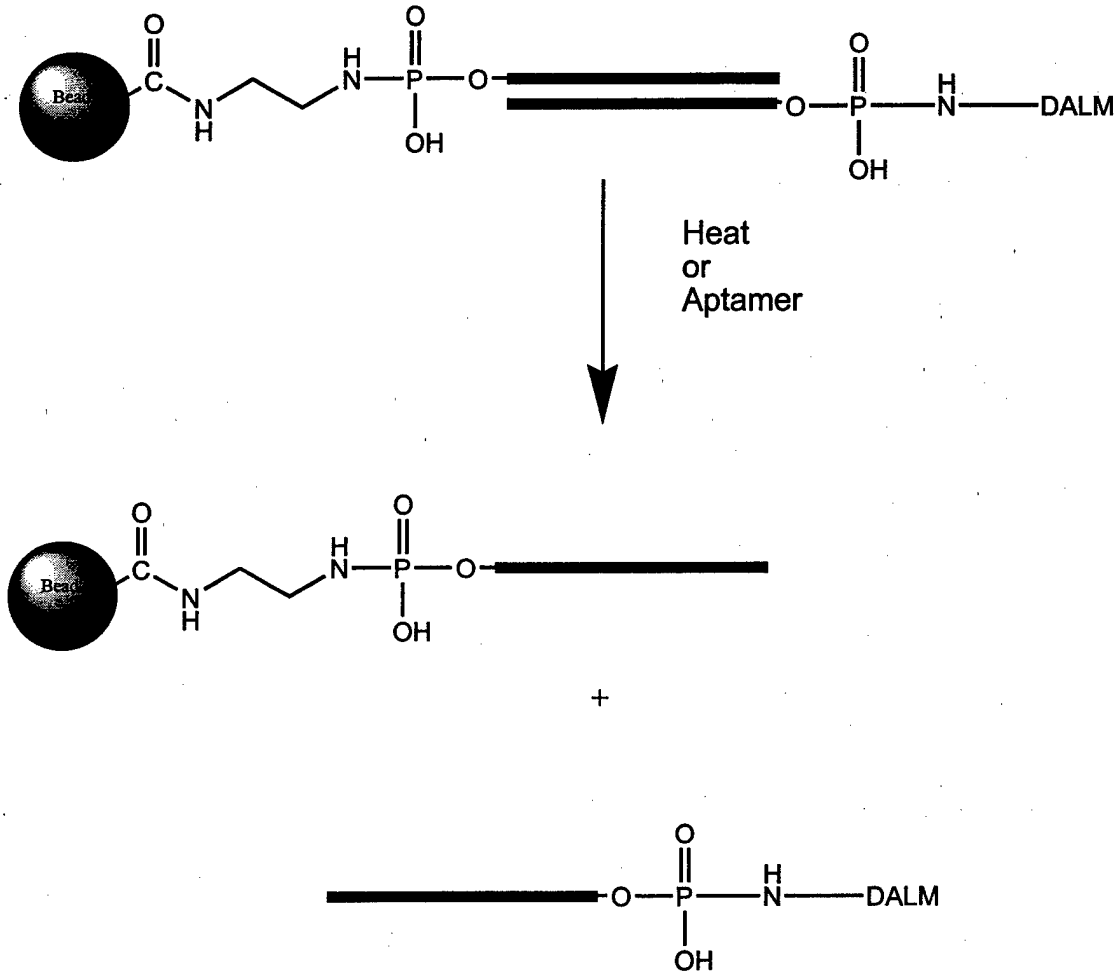
5.1 Figures showing the chemistry discussed in the methods section.

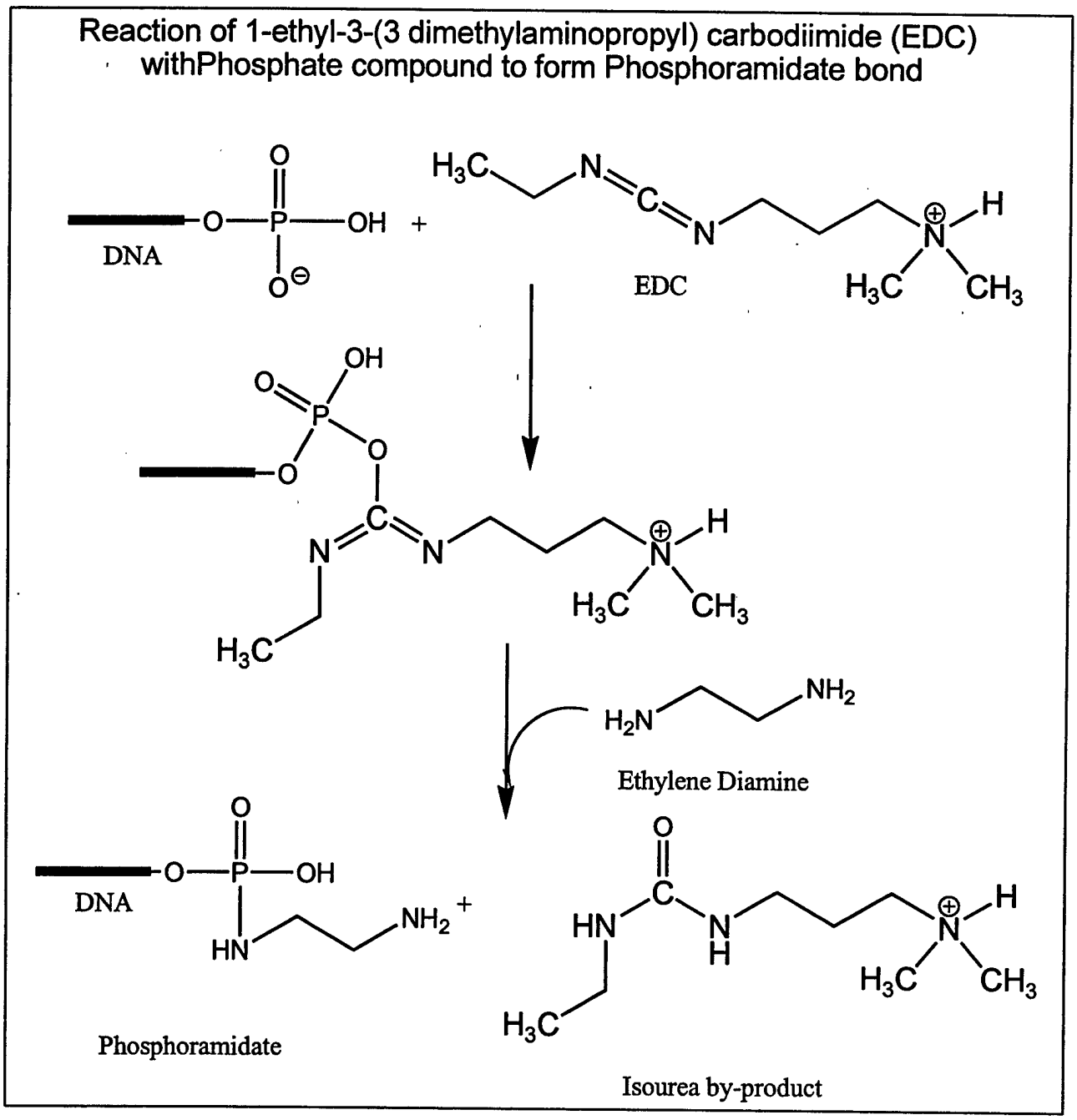



Synthesis of Amino DALM
H2N
NH2
+
HOOC — DALM
Ethylene Diamine
EDC
H
N
DALM
H2N
O
1. Dialysis
2. Ultrafiltration
H
N
DALM
H2N
O


Addition of DNA and DALM to Beads
Bead
O
OH
+
H_2N
NH_2
EDC
C
N
H
dsDNA
P
Amino-DALM
DALM

Effect of Heat or Binding of Aptamer on Bead Complex





5.2 References

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Kiel, J.; O'Brien, G.; Dillon, J.; Wright, J., "Diazoluminomelanin: A Synthetic Luminescent Biopolymer", *Free Rad. Res. Comms.* **8**, 115 (1990).

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